REVERSE MICELLAR MOBILE PHASES FOR NORMAL PHASE CHROMATOGRAPHY

By

MARIA A. HERNÁNDEZ TORRES

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

1983

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation to my research director, Dr. John G. Dorsey, for his invaluable assistance, guidance and encouragement throughout the course of this project.

Also, my special thanks are extended to Mr. John S. Landy, for his help in the initial setting of the instrument and his continuous assistance.

I would like to thank my colleagues in Dr. Dorsey's research group for their friendship and daily encouragement.

I would also like to thank my family and friends for their patience and continuous support.

Finally, I would like to mention Patty Hickerson for her promptness and accuracy in typing the final draft of this thesis.

TABLE OF CONTENTS

Abstract of Thesis Presented to the
Graduate School of the University of Florida
in Partial Fulfillment of the Requirements for the
Degree of Master of Science

REVERSE MICELLAR MOBILE PHASES FOR
NORMAL PHASE CHROMATOGRAPHY

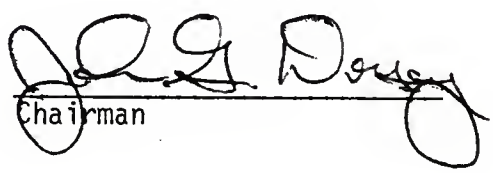
By

María A. Hernández Torres

December 1983

Chairman: John G. Dorsey
Department: Chemistry

Reverse micelles, formed in hexane, can be used in place of polar modifier to control the strength and selectivity of normal phase mobile phases for liquid chromatography. The effect of surfactant concentration (Aerosol OT) on retention of various solutes in a normal phase liquid chromatographic system was investigated. Surfactant concentration above the critical micelle concentration range was used to observe the change in capacity factor, k', as the water content in the mobile phase was varied. Small changes in k' values were obtained at low water content in the micellar mobile phase. Finally, the column efficiencies for dry hexane, 5/95 isopropanol/hexane and 5×10^{-2} M AOT in hexane mobile phases were compared for Ultrasil-NH_2 and Ultrasphere-Si columns.



Chairman

CHAPTER I

INTRODUCTION

A great deal of research has been put into the improvement of both the stationary and mobile phases of high performance liquid chromatography (HPLC) to achieve better separations.[1-3]

This study is limited to normal phase HPLC, for which the retention of the normal phase separation increases with increasing solute polarity and decreases with increasing mobile phase polarity. The solute retention mechanism is dominated by interaction of polar stationary phase sites with polar solutes.

The advantages of normal phase chromatography are summarized by Abbot[4] as follows:

1) ability to separate highly hydrophilic species which cannot be retained on reverse phase

2) use of predominantly organic solvent-based mobile phases, avoiding silica dissolution problems experienced in reversed phase

3) ability to differentiate solutes based on differences in hydrophilic structure rather than hydrophobic structure

4) compatibility of organic phase with molecules having either low stability or aggregation problems in aqueous phases

5) lower viscosity of organic mobile phases, resulting in lower operating pressures

6) mobile phase volatility, allowing simpler, more efficient concentration and transfer steps in off-line fraction collection and structural characterization

7) ability to separate isomers which is of great significance in natural product and pharmaceutical chemistry

8) ability to obtain class separations which cannot be obtained in reversed phase

9) greater compatibility of organic mobile phase with on-line coupling

10) availability of wide range of organic solvents to capitalize on special solvent effects

11) availability of a wide range of stationary phase selectivities, which can be utilized to obtain high separation performance in multi-stage chromatography.

Normal phase chromatography typically encompasses adsorption chromatography on silica and partition chromatography on cyano and amino bonded phases.

Adsorption may be defined as the concentration of solute molecules at the interface of two immiscible phases. In liquid-solid adsorption chromatography, the mobile phase is a liquid, while the stationary phase is a finely divided, usually porous, solid. The atoms in the bulk of the solid are subjected to equal forces in all directions, whereas the surface atoms experience unbalanced forces which can attract molecules from the surrounding solution to restore the balance.

In a multicomponent system, selective adsorption occurs due to competition between the solutes and the mobile phase for the surface. It is governed by the differences in the strengths of the adsorption forces between the adsorbent and the adsorbates. In general, polar compounds are more strongly adsorbed by polar solids than are nonpolar compounds. Also, adsorption of a polar compound is enhanced in a nonpolar medium, but reduced in a polar medium due to increased competition of the mobile phase for the surface.

The partition process of a solute occurs between a liquid mobile phase and a liquid absorbed on, or chemically bonded onto, a porous support. Classical liquid-liquid chromatography involves the partition of the sample components between a liquid stationary phase and the liquid mobile phase. The separation occurs due to differences in the solubilities of the sample components in the two liquid phases. Since liquids which are practically immiscible will usually still have at least ppm solubility in each other, the mobile phase must be presaturated with the polar phase to prevent gradual column stripping and loss of separating power. As a result, column packings have been developed in which the stationary phase is permanently bonded to the support by chemical bonding. Bonded phase packings are prepared by the reaction of the surface Si-OH groups of the support particles with various reagents[5] (Figure 1). These siliceous supports are prepared with a variety of functional groups, ranging from very polar to nonpolar, resulting in widely diverse selectivities. The amino($-NH_2$) and cyano(-CN) functional groups are polar in nature, and they are commonly used with low polarity mobile phases for normal phase separations.

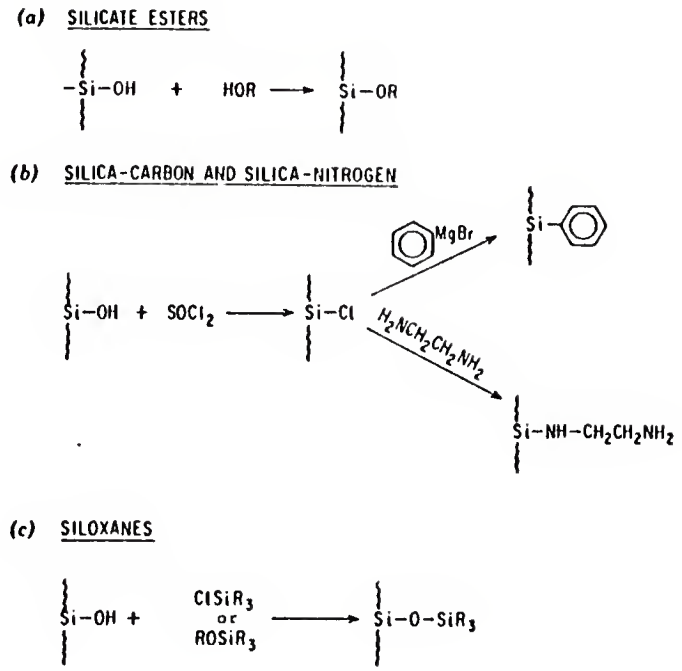


Figure 1. Reactions for preparing bonded-phase packing (from[5]).

It has been recognized that retention on active stationary phases such as silica and alumina with nonpolar and moderately polar eluents is strongly influenced by the water content of the eluent.[6-9] The surface of the silica gel is covered with Si-OH and Si-O-Si groups which can interact with the sample molecules. There are two kinds of hydroxyl groups covering the silica gel surface: free (OH) and reactive (HOH) hydroxyl groups. The reactive hydroxyl groups are very strong bonding agents which may permanently adsorb polar sample and water molecules onto the gel, giving nonreproducible results. Since the water content of the absorbent markedly affects relative capacity ratio (k') values and band migration rates, water content must be held constant for repeatable separation.

Surfactants are amphiphatic molecules which have distinct hydrophobic and hydrophilic regions. Over a narrow concentration range, defined as the critical micelle concentration (CMC), surfactants dynamically associate to form large molecular aggregates. In nonpolar solvents, these have been termed reversed or inverted micelles, since their polar groups are concentrated in the interior of the aggregate while their hydrophobic groups extend into and are surrounded by the bulk apolar solvent (Figure 2). In aqueous solution, the micelle is a compact roughly spherical body with a liquid-like hydrocarbon core. The polar head groups are at the surface (Figure 3). Micellar systems have been introduced in reverse phase HPLC, and an improvement in the separations has been reported.[2,3,10,11] When using micellar mobile phases, solutes do not partition to the bulk solvent but rather to the discrete aggregates creating a unique separation mechanism:

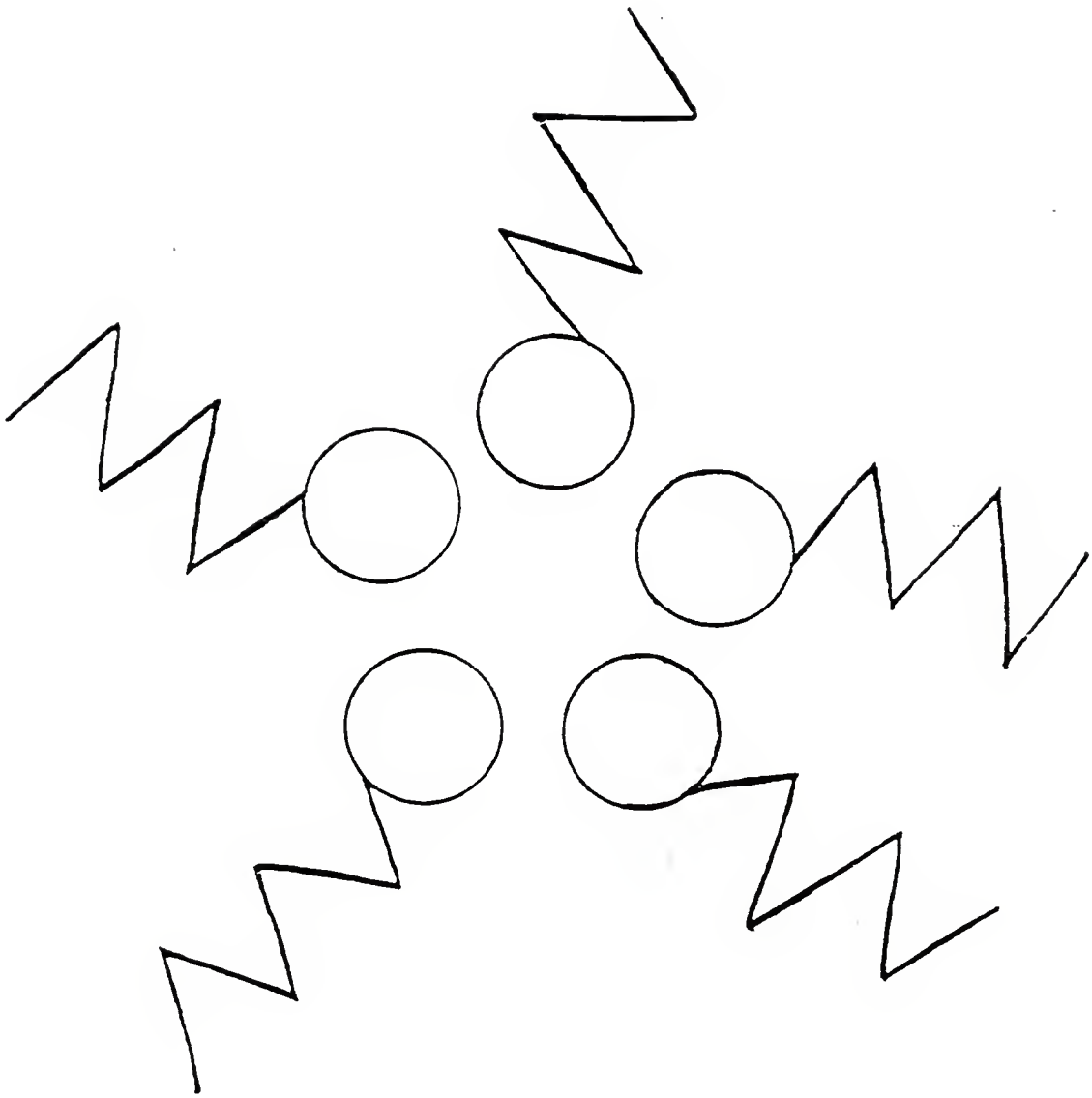


Figure 2. Reverse micelle representation. The ionic head group (O) points toward the center of the micelle, and the hydrocarbon chain (∿∿) surrounds the central ionic core (from [12]).

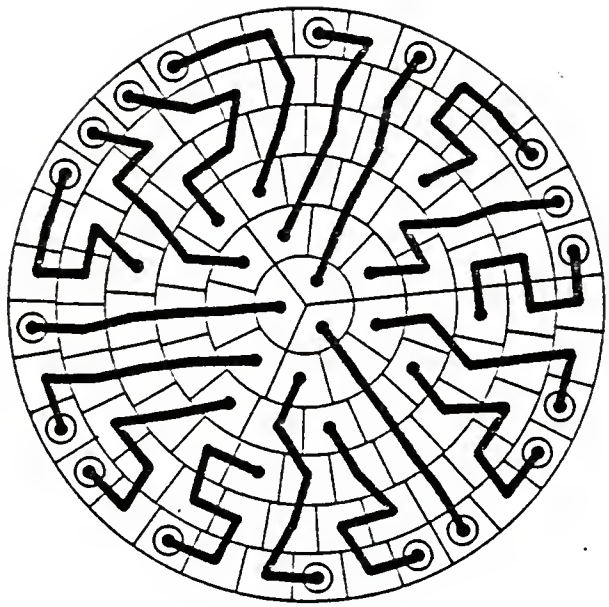


Figure 3. Dill-Flory's representation of a normal micelle. The ionic head groups are indicated by the circles, and the hydrocarbon chains are pointing toward the center of the micelle (from[12]).

pseudo phase liquid chromatography. Some of the advantages of the micelle system in RPHPLC are:[13]

1) many hydrophobic, amphiphilic, and even hydrophilic molecules interact differently with the micelles, allowing for a wide range of applications

2) low cost

3) nontoxic

4) non-UV absorbing

5) simultaneous separations of hydrophobic and hydrophilic solutes are accomplished without aqueous organic gradients

6) the addition of solutes to the mobile phase to control ionic strength, pH, and buffer capacity will not produce solubility problems

7) a relatively small change in the amount of surfactant will essentially give a different mobile phase.

Our studies are directed mainly to observe the behavior of solutes in reverse micellar systems. Solute's retentions as a function of surfactant concentration are examined. Also, this study attempts to find whether the water in the chromatographic system will be entrapped so that the retention of the solutes in the normal phase HPLC will not be changed. The best column efficiency is sought among all the conditions studied.

CHAPTER II

THEORY

Due to the new findings and applications of reversed or inverted micelles in the course of the last few years, the scientific interest with regard to nonpolar detergent solutions has extensively increased.[14]

The obvious difference between micelles in polar and nonpolar surfactant solutions is their mutual structural reversion. In nonpolar solvents, the inverted micelle is visualized to be built up by a polar core covered by hydrocarbon tails (vide supra). Inverted micelles have moderate aggregation numbers which contrast the large micellar aggregates in aqueous surfactant solutions, e.g., for 4.9×10^{-4} M of Aerosol OT in pentane at 25°C the mean aggregation number ($\bar{n}$) is 15, while for 8.2×10^{-3} M of sodium n-dodecylsulfate (SDS) in aqueous solution at 25°C $\bar{n}$ is 64.[14-16]

In the formation of a micelle in apolar media, there seems to be a general agreement with regard to the existence of premicellar aggregates. The monomer $\rightleftarrows$ n-mer type association is unlikely to represent the behavior of surfactants.[16] The most universally used treatment, the multiple equilibrium model, assumes the stepwise formation of aggregates in an indefinite association: monomer $\underline{\underline{\quad k_1 \quad}}$ dimer $\underline{\underline{\quad k_2 \quad}}$ trimer $\underline{\underline{\quad k_3 \quad}} \cdots \underline{\underline{\quad k_n \quad}}$ n-mer, where the distribution of the different aggregates depends on the stoichiometric surfactant

concentration. The higher the concentration of the surfactant, the larger the aggregates. Under this treatment, the CMC can be defined as the concentration of aggregates within a narrow distribution centering around the average micellar size. On the other hand, it can be assumed that there is no unique concentration at which the existence of micelles becomes detectable; therefore, it is unjustified to speak of one concentration, or even a narrow range of concentration, describing the CMC of surfactants in hydrocarbon solutions.[17]

The driving force behind the process of aggregation and micellization of surfactants in hydrocarbon media is essentially due to dipole-dipole interactions between polar heads of the amphiphilic molecules.

One important property of the micellar system is the detergency or the ability of surfactant molecules to take up (solubilize) polar material, i.e., water in the polar core of the inverted micelles.

There are a large number of parameters which influence and even determine the extent of the solubilization of the polar material. These parameters include the structure of the surfactant, the physicochemical property of the solubilizate, temperature, pressure, electric fields and cosurfactants.

Considerable amounts of water can be solubilized by reverse micelles. This surfactant solubilized water is often called waterpool. Initial water molecules are bound to the polar head groups, and their motion is restricted. Additional water molecules occupy the core of the micelle, and their properties resemble bulk water. A rapid exchange takes place between these two kinds of water molecules.

The effective polarity, acidity and microscopic viscosity of the water pools are expected to be substantially different from those in bulk water.

Polar substrates are expected to be localized in water pools; therefore, controlled amounts of surfactant-entrapped water in non-polar solvents provide a unique medium for interactions of polar substrate.

CHAPTER III

EXPERIMENTAL

Instrumentation

A Spectra-Physics 8700 (Santa Clara, California) liquid chromatograph equipped with a model 7125 Rheodyne sample injection valve with a 10 microliter loop, a model 153 Beckman UV detector (254 nm) with an 8 microliter flow cell, and a model 1210 Linear strip chart recorder were used. The columns used were: an Altex (Berkeley, CA) Ultrasphere-Si (15 cm x 4.6 mm ID) 5 μm particle size and an Altex Ultrasil-NH_2 (25 cm x 4.6 mm ID) 10 μm particle size. Both columns were thermostatted at 30°C ± 0.2 using a water jacket and a Haake D1 water circulator.

Reagents

HPLC grade n-hexane (Fisher Scientific) was used as the solvent; it was dried using Linde Molecular Sieves (Union Carbide) type 3A.

Reagent grade 2-propanol (Fisher Scientific) was used as organic modifier and to wash the columns.

Reagent grade Aerosol OT (dioctyl sodium sulfosuccinate, Fisher Scientific) was used without further purification; it was dissolved in hexane and then filtered with a Rainin solvent filtration apparatus through a 0.45 μm membrane filter.

Water used in the mobile phase was purified using a Barnstead Nanopure System (Symbron Corporation).

The solutes were used without further purification, and all the solute solutions were prepared in n-hexane. The solutes were: 2,4-dinitrotoluene, naphthol (Matheson Coleman & Bell) and phenol (Mallinckrodt).

Reagent grade n-pentene (J.T. Baker) was used as an unretained compound for the column's void volume calculation.

Procedure and Calculations

The appropriate weight of surfactant (Aerosol OT) was weighed and dissolved in hexane. The concentration range was from 1×10^{-4} M to 1×10^{-1} M with alternate increments of 5x and 2x, respectively. The columns were thermostated at 30°C. A silica gel precolumn was used before the injector to prevent the silica in the column from being dissolved by the surfactant. The columns were washed with 2-propanol, and rinsed and stored in n-hexane.

A stock surfactant solution was prepared of 0.50 M, and aliquots of 50 ml were taken and diluted with n-hexane to 5.0×10^{-2} M for the study of the influence of mobile phase water content on the retention time of the solutes. In each of the six 5.0×10^{-2} M Aerosol OT solutions, the volume percentage of water was varied (0%, 0.01%, 0.02%, 0.05%, 0.10%, 0.25%).

The capacity factor, k', was used to compare the retention data of the solutes and was calculated using the following equation:

$$k' = \frac{V_r - V_o}{V_o} \qquad (1)$$

where Vr = the retention volume of a given solute and is calculated as the product of flow rate times retention time (ml), and V_o = the

column's void volume (ml). n-Pentene, assumed to be an unretained solute, was used to calculate the void volume of the column.

The efficiencies of the columns were compared using the reduced plate height, h, given by:

$$h = \frac{L}{N \cdot dp} \qquad (2)$$

where L = the column length, dp = the particle diameter, and N = the plate number. The plate number, N, was calculated as follows:[18]

$$N = \frac{41.7 \left(\frac{t_R}{w_{0.1}}\right)^2}{B/A + 1.25} \qquad (3)$$

where t_R = the retention time of the compound (cm), $w_{0.1}$ = the peak width at 10% of the peak height (cm), and B/A = the asymmetry factor.

CHAPTER IV

RESULTS AND DISCUSSION

Retention Changes with Surfactant Concentration

Figures 4 and 5 show the behavior of phenol and naphthol in hexane/Aerosol OT mobile phases. These graphs show two linear components, one above and one below the CMC. Since the formation of micelles in nonpolar solvents occurs in sequential steps, it is difficult to assign the CMC to one point. The intersection of the two linear components was found to be 3.5×10^{-3} M AOT in hexane. Therefore, the concentration range from 1.0×10^{-3} M to 5.0×10^{-3} M AOT in hexane was assigned as the CMC, in agreement with the formation of reverse micelles as the multiple equilibrium model postulates. In both columns, Ultrasphere-Si and Ultrasil-NH_2, the first large change in k' was observed in the same range of concentration, the CMC range. In other words, the same value of CMC range for AOT in hexane was obtained for the two different columns and two different solutes. Table I shows mean aggregation numbers and critical micelle concentrations of Aerosol OT in different hydrocarbon solvents.

At concentrations above the CMC range, the slope of the four curves are slightly negative; with the Ultrasphere-Si column having a greater negative slope. This can be attributed to the fact that the silica surface has silanol groups that interact with polar compounds

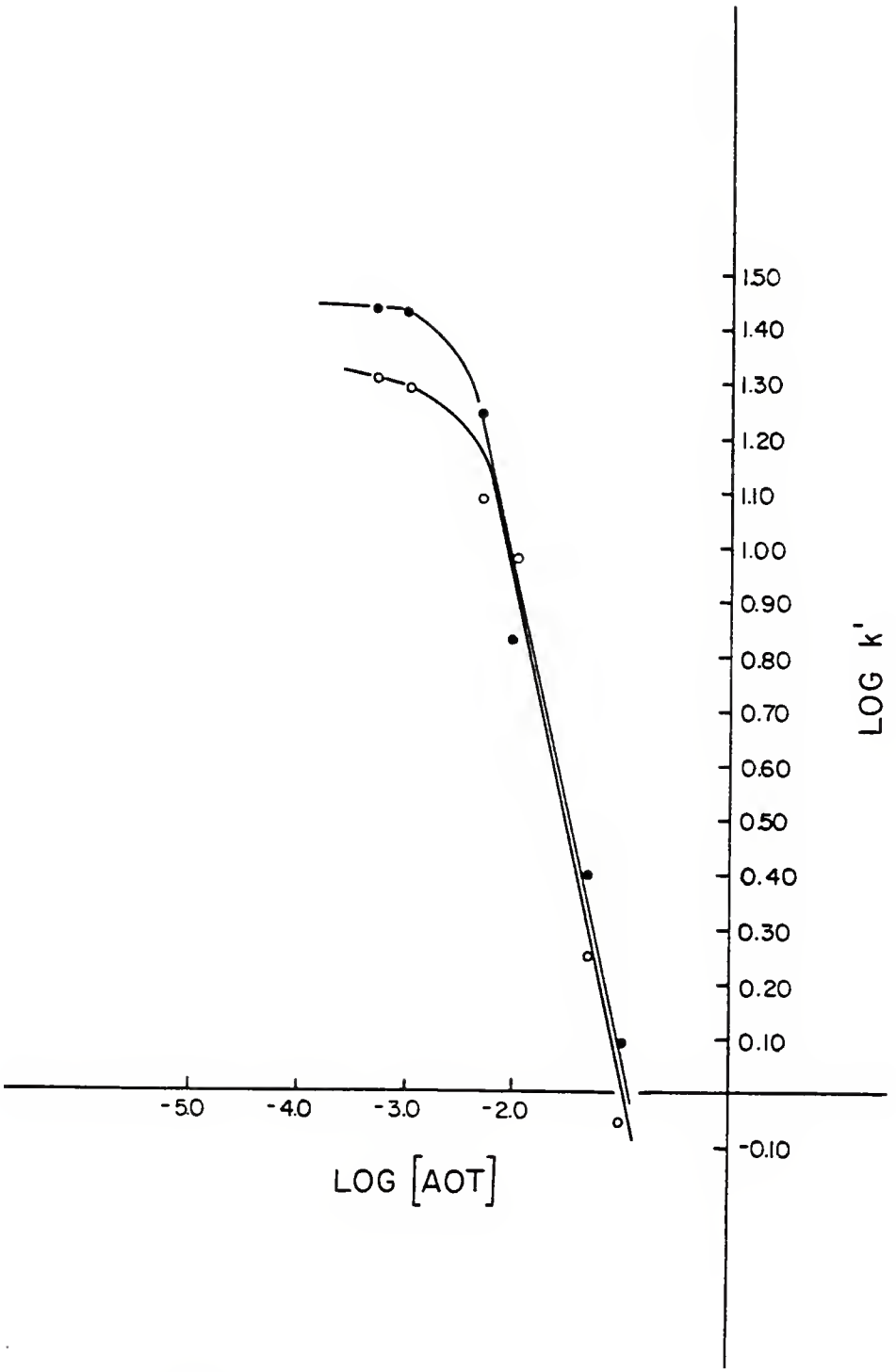


Figure 4. Effect of surfactant concentration on retention using Ultrasphere-Si column. (0) naphthol, (●) phenol.

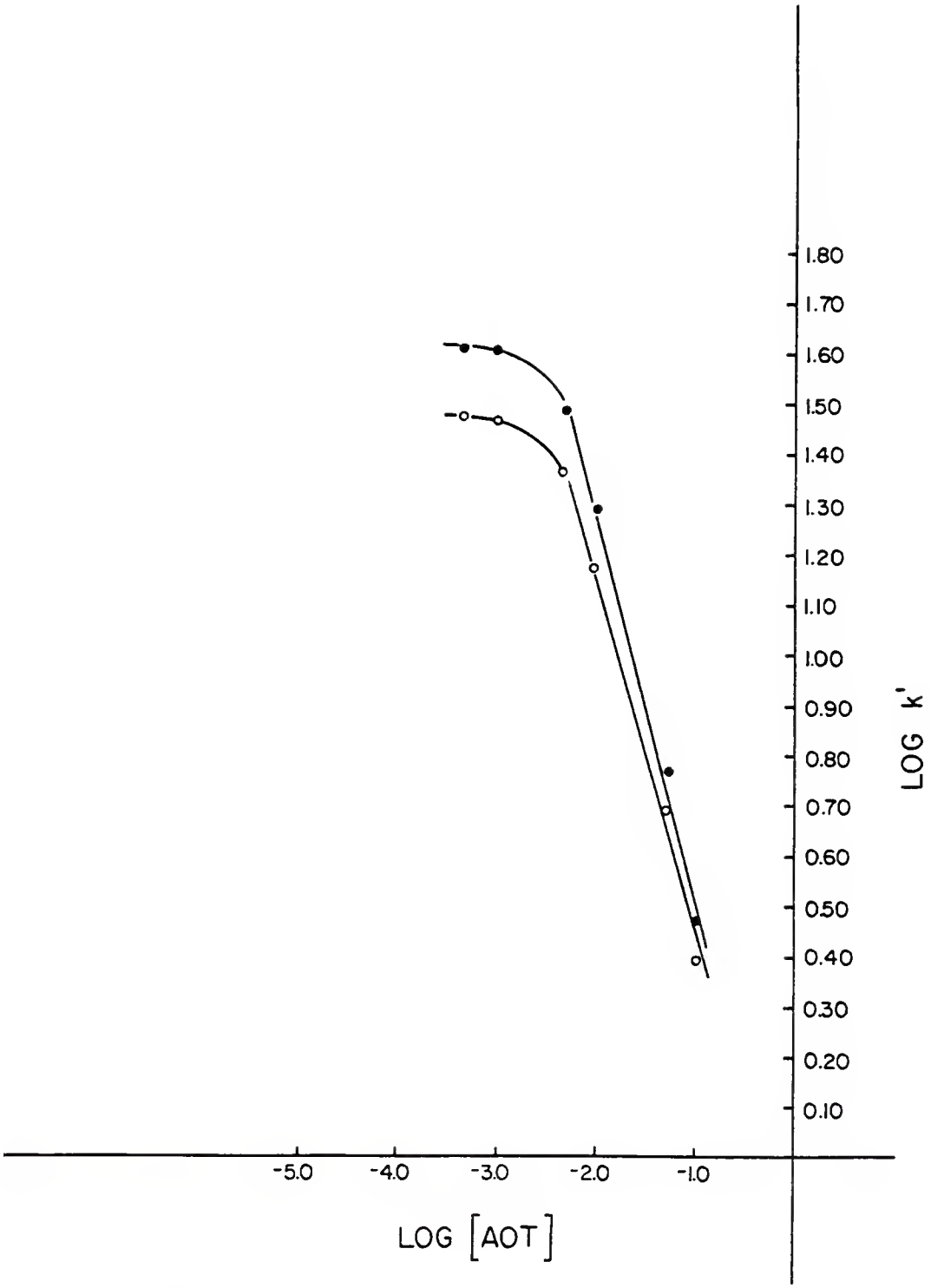


Figure 5. Effect of surfactant concentration on retention using Ultrasil-NH_2 column. (●) naphthol, (○) phenol.

Table I

Mean Aggregation Numbers ($\bar{n}$) and CMCs of Aerosol OT in Different Hydrocarbon Solvents (from [14])

Solvent	Temperature (°C)	Technique[a]	CMC (M)	$\bar{n}$
Cyclohexane	37	VPO	3.9×10^{-4}	17
	28 ± 4	LS	1.3×10^{-3}	56
Benzene	37	VPO	3.5×10^{-4}	13
	RT	PA	2.2×10^{-3}	-
	28 ± 4	LS	2.7×10^{-3}	23.6
	20	TCNQ	2.0×10^{-3}	-
CCl_4	25	VPO	1.6×10^{-4}	17
	37	VPO	4.0×10^{-4}	17
	20	TCNQ	6.0×10^{-4}	-
Pentane	25	LS	4.9×10^{-4}	15

[a] VPO: vapor pressure osmometry
LS: light scattering
PA: positron annihilation
TCNQ: solubilization of 7,7,8,8-tetracyanoquinodimethane

in a very strong way. Also, the polar heads of the surfactant can interact with these active sites, and retention of polar compounds will decrease as the number of active sites available is reduced by the presence of free surfactant. In the Ultrasil-NH_2 column, the interaction of the polar surfactant head groups with the amino stationary phase groups is less strong; therefore, the concentration of free surfactant in the mobile phase for this column affects the retention of polar compounds to a lesser extent. Recently, Deming and Tang proposed that addition of surfactant to the eluent reduces the interfacial tension and thus decreases the retention time of the injected samples.[19]

Looking at Table II, one can observe a big change in k' value going from dry hexane to 5×10^{-4} M AOT in hexane mobile phases. Two possible reasons for this change are (1) before the CMC range, the surfactant is in a monomeric form, and the polar heads compete for active sites in the stationary phase, and (2) the formation of small aggregates at very low concentration of surfactant that can interact with solutes. For both points, there is a decrease in the number of active sites available at the stationary phase; therefore, a decrease in the retention time of solutes is observed.

Above the CMC range, the k' of naphthol and phenol decrease very rapidly as the formation of aggregates increases. The reason for this behavior is that in a reverse micellar system a polar solute will partition to the polar core of the micelle spending less time interacting with the stationary phase. This separation mechanism with reverse micelles is similar to the one discussed by Armstrong and Nome[20] for

Table II

k' Value for Phenol and Naphthol in Dry Hexane and 5×10^{-4}M AOT in Hexane for Ultrasphere-Si and Ultrasil-NH_2 Columns

Column	Mobile Phase	k' phenol	k' naphthol
Ultrasphere-Si	dry hexane	72.53	47.50
	5×10^{-4} M AOT in hexane	27.89	20.30
Ultrasil-NH_2	dry hexane	47.52	53.75
	5×10^{-4} M AOT in hexane	29.44	40.87

micellar systems in reversed phase HPLC, or pseudo phase liquid chromatography. In normal phase chromatography, the solvent strength is varied in order to optimize k'. Increasing the strength of the mobile phase by increasing the solvent polarity, a decrease in sample k' value is observed. Preferred solvents used in normal phase to increase the strength of the mobile phase are acetonitrile, ethanol, ethylacetate, $CHCl_3$, tetrahydrofuran and isopropyl ether.

Comparing micellar and organic mobile phases for normal phase HPLC, one can say that (1) Organic mobile phases have somewhat lower viscosity than micellar mobile phases, resulting in lower operating pressures. For example, at a flow rate of 1 ml/min., 30°C, the pressure for 5/95 isopropanol/hexane mobile phase was 302 psig vs. 350 psig for 5×10^{-4} M AOT in hexane mobile phase for Ultrasphere-Si column. Table III shows the viscosity values for most common solvents used in normal phase HPLC. (2) Surfactants are cheaper than the HPLC grade organic solvents; an approximate cost per gallon of organic solvent is thirty dollars vs. twenty dollars for 500 g of Aerosol OT (Table III). (3) Surfactants are non-toxic vs. some of the most commonly used organic modifiers, for example tetrahydrofuran. (4) Micellar mobile phases offer a different selectivity for separations of compounds, and (5) For both mobile phases, a UV detector can be used. The molar absorptivity coefficient for Aerosol OT was calculated to be 0.9986 L/mole cm at a wavelength equal to 254 nanometers (Table III).

Table III

Viscosity, UV Cutoff and Price per Gallon for Most Commonly Used Solvents in Normal Phase HPLC From[21,22]

Solvent	UV Cutoff (nm)	Viscosity (cP, 25°C)	Price per Gallon
Hexane	195	0.30	28.65
i-Propyl ether	220	0.38	27.85
Tetrahydrofuran	212	0.46	47.15
i-Propanol	205	1.9	24.80
Chloroform	245	0.53	29.85
Acetonitrile	190	0.34	55.85
Ethanol	210	1.08	26.75
Ethylacetate	256	0.43	26.05

Efficiency

In looking for an optimum separation, one which gives adequate sample resolution with a minimum of time and effort, one of the parameters to be considered is column efficiency. The number of theoretical plates, N, was calculated in order to compare the performance of the system under the different experimental conditions studied. Foley and Dorsey's equation was used to calculate the number of theoretical plates.[18] (vide supra) This equation was preferred over the most commonly used,

$$N = 5.54\ (t_r/w_{0.5})^2 \qquad (4)$$

because the former corrects for the asymmetry of skewed peaks giving more accurate values for N. The reduced plate height was calculated by

$$h = H/dp \qquad (5)$$

where H is the plate height, and dp is the particle diameter.

Table IV shows the theoretical plate numbers, plate heights, reduced plate height and asymmetry ratios for phenol in 5×10^{-2} M AOT in hexane, 5/95 isopropanol/hexane and dry hexane mobile phases for Ultrasphere-Si and Ultrasil-NH_2 columns.

Better efficiencies were observed for 5/95 isopropanol/hexane mobile phase for both columns. The reason for smaller values of N in 5×10^{-2} M AOT is the higher viscosity of the micellar solution, having smaller diffusion coefficients, therefore, slower mass transfer. This slow mass transfer results from the slower rate of movement of a polar sample between the stationary phase and the interior of the

Table IV

Theoretical Plate Numbers (N), Plate Heights (H, mm), Reduced Plate Heights (h) and Asymmetry Ratios (B/A) for Phenol in 5×10^{-2} M AOT in Hexane, 5/95 Isopropanol/Hexane and Dry Hexane Mobile Phases for Ultrasphere-Si and Ultrasil-NH_2 Columns

	5×10^{-2} M AOT in Hexane	5/95 Isopropanol/Hexane	Dry Hexane
Ultrasphere-Si			
N	3301	8174	3987
H (mm)	0.0454	0.0184	0.0376
h	9.08	3.68	7.40
B/A	1.50	1.14	2.02
Ultrasil-NH_2			
N	902	2409	-
H (mm)	0.277	0.104	-
h	27.7	10.4	-
B/A	2.18	1.22	-

micelle. Also, higher viscosity in the interior of the micelle in comparison of the bulk solution contributes to slow the adsorption-desorption interchange.

Greater values for asymmetry ratio in micellar system were obtained in accordance with the N values. Optimization of the micellar system has to be done in order to be a feasible medium for separation.

For Ultrasphere-Si columns, higher values of N were calculated for the three systems studied. This can be explained in terms of a smaller particle diameter, 5 μm vs. 10 μm for Ultrasil-NH_2. It seems that the fact of smaller particle diameter dominates over the longer column for Ultrasil-NH_2 and stronger active sites in Ultrasphere-Si.

Lower efficiency was observed for phenol in Ultrasphere-Si in dry hexane mobile phase. This was expected since it is generally accepted that with a dry mobile phase, at low water contents, much lower efficiencies will be obtained because of the strong active sites of the adsorbent surface, leading to slow adsorption-desorption kinetics.[6] The theoretical plate number for phenol in Ultrasil-NH_2 could not be calculated because of the long retention (more than three hours).

In Szepezy, Combellas, Claude and Rosset's paper,[6] very low and rapidly changing values of efficiency at low water contents were pointed out, indicating the necessity for selection and control of the water content in the mobile phase. For this reason, the next part of this experiment was performed.

Influence of Water Content on Retention

One of the disadvantages in using normal phase chromatography is the changes in the chromatographic characteristics (capacity ratio, selectivities, efficiencies) with increasing water content of the mobile phase.

In order to observe the influence of the water content on retention, plots of k' vs. volume percent of H_2O in 5×10^{-2} M AOT/hexane were drawn for both columns. The surfactant concentration in the mobile phase was chosen above the CMC range to assure the existence of micelles.

Figures 6 and 7 show the behavior of phenol, naphthol, and 2,4-dinitrotoluene in Ultrasil-NH_2 and Ultrasphere-Si columns. A smooth change in k' was observed at low water concentrations. For the amino column, only a slight change was noticed; this can be attributed to the fact that the silica surface is more active. (vide supra)

Retention values are quite sensitive to small changes in mobile phase with low water concentration. In actual practice, it is difficult to avoid small changes in mobile phase water content, for several reasons:[23]

-The water contents of the starting (supposedly dry) solvents that comprise the mobile phase vary somewhat.

-The water is readily picked up or lost to the atmosphere, depending on its humidity and water content of the solvent.

-Further changes in mobile phase water content can occur as a result of contact with the walls of intermediate containers, the solvent reservoir, and so on.

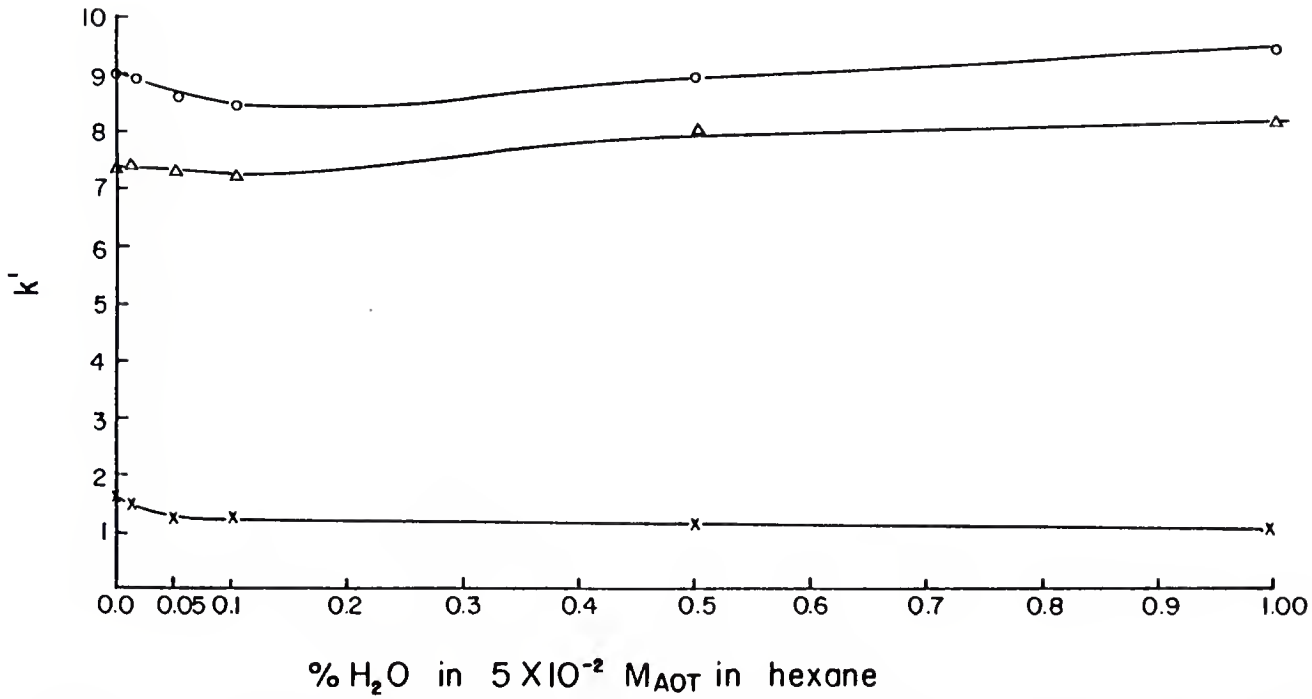


Figure 7. Effect of water content variation on retention using an Ultrasil-NH_2 column. (O) naphthol, (Δ) phenol and (X) 2,4-dinitrotoluene

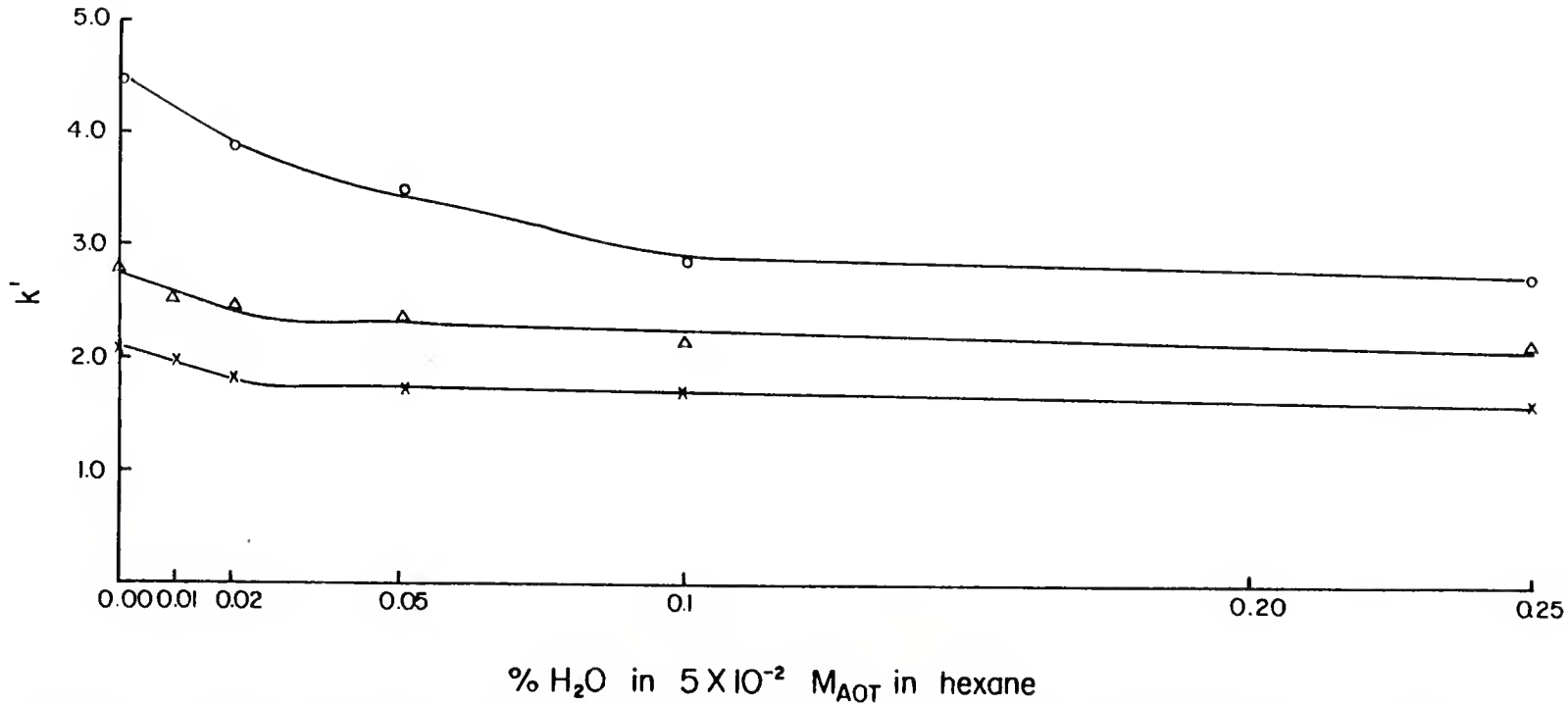


Figure 6. Effect of water content variation on retention using an Ultrasphere-Si column. (O) 2,4-dinitrotoluene, (Δ) phenol and (X) naphthol

The importance of controlling the water content at low water concentration in order to obtain reproducibility of k' values has been pointed out by many authors.[6,7,23] Szepezy, Combellas, Claude and Rosset demonstrated in their paper[6] that for silica packing, a small increase in water content corresponds to a large decrease in k' values. Their k' values change up to one order of magnitude for dibutylphthalate varying from 100 to 600 ppm of water.

The solubility of water in hexane at 20°C, 1 atm, is 0.0111 g water per 100 g of hexane, or 0.0073 percent V/V.[24]

Using reversed micellar system, a maximum change of 38 percent in k' was observed at low water compositions (Table V). This small change in k' values as the water content is increased can be explained by the fact that reverse micelles tend to solubilize a large amount of water in their interior. The first molecules of water are attached to the counterion (sodium) which connects, via two hydrogen bridges to two sulfonate molecules, forming a trimer. Additional water molecules occupy the core of the micelle, and polar substrates are expected to be localized in this water pool. Small changes in the amount of water do not affect the retention, since almost all the water in the system is restricted to the interior of the micelle. Instead, it has been concluded that small amounts of water are a prerequisite to the formation of closed, concentration independent surfactant aggregates.[25] Large amounts of water cause the micelle to swell, increase the mean aggregation number and to assume a different shape; therefore, it will be interesting to find out the amount of water that the micelle can hold without drastic changes in the k' value.

Table V

Percent of k' Values Changes as the Water Content Varies from 0.0 to 0.25 Percent for Ultrasphere-Si and from 0.0 to 1.0 Percent for Ultrasil-NH_2 Columns in 5 x 10^{-2} M AOT in Hexane Mobile Phase

Column	% Change Phenol	% Change Naphthol	% Change 2,4-Dinitrotoluene
Ultrasphere-Si	11.28	10.14	32.53
Ultrasil-NH_2	24.03	19.81	38.27

CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

Reverse micellar systems are a promising mobile phase for normal phase HPLC, as normal micellar systems have been shown to be in reversed phase HPLC. As the concentration of surfactant in the mobile phase is increased, the retention times of polar compounds decrease. Drastic reductions in k' values for surfactant concentrations above the critical micellar concentration range were observed. Only small changes in capacity ratio values for polar solutes were obtained at low water contents in the mobile phase. Due to the fact that the amount of water in the mobile phase is critical in order to obtain reproducible retention values in normal phase chromatography, an addition of a small amount of water in micellar mobile phases may be a feasible means to obtain reproducible k' values. Further studies should be done in this area. It will be interesting to look for the maximum amount of water than can be held by the micellar mobile phase without an abrupt change in k' value.

Lower efficiencies were obtained for micellar mobile phases because of the higher viscosity of these mobile phases, leading to slower mass transfer. Raising the temperature or adding small amounts of polar organic modifiers[3] may be a good approach to improving efficiencies for reverse micellar mobile phases, and should be investigated.

The selectivity (α), or relative retention of solutes, has a considerable effect on resolution. The value of α should be as large as possible, other factors being equal. The use of a micellar mobile phase and changing temperature are two options that can be studied in the future in order to increase α, thereby improving resolution.

A similar study can be performed using a cyano column and more polar solutes in order to clarify the retention mechanism of polar compounds in reverse micellar systems.

Aerosol OT is an anionic surfactant. A study of different anionic surfactants, cationic surfactants and nonionic surfactants will give different types of aggregation systems from which the separation mechanism can be observed.

REFERENCES

1. R.E. Majors. Recent advances in HPLC packing and columns. J. Chromatogr. Sci. 18, 488-511 (1980).

2. D.W. Armstrong and S.J. Henry. Use of an aqueous micellar mobile phase for separation of phenols and polynuclear aromatic hydrocarbons via HPLC. J. Liq. Chromatogr. 3, 657-662 (1980).

3. J.G. Dorsey, M.T. DeEchegaray and J.S. Landy. Efficiency enhancement in micellar liquid chromatography. Anal. Chem. 55, 924-928 (1983).

4. S.R. Abbot. Practical aspects of normal-phase chromatography. J. Chromatogr. Sci. 18, 540-550 (1980).

5. L.R. Snyder and J.J. Kirkland. "Introduction to Modern Liquid Chromatography," 2nd Ed., John Wiley and Sons, New York, 1979, pp. 272-277.

6. L. Szepezy, C. Combellas, M. Claude and R. Rosset. Influence of water content of the mobile phase on chromatographic performance in adsorption chromatography. J. Chromatogr. 237, 65-78 (1982).

7. L.R. Snyder. "Principles of Adsorption Chromatography," Marcel Dekker, Inc., New York, 1968, pp. 157-159.

8. J.J. Kirkland. "Modern Practice of Liquid Chromatography," John Wiley and Sons, New York, 1971, pp. 215-218.

9. R.W. Yost, L.S. Ettre and R.D. Conlon. "Practical Liquid Chromotography," Perkin Elmer Corporation, New York, 1980, pp. 60-62.

10. D.W. Armstrong. Pseudophase liquid chromatography: applications to TLC. J. Liq. Chromatogr. 3, 895-900 (1980).

11. D.W. Armstrong. Application of pseudophase liquid chromatography (PLC). Amer. Lab. 13, 14-20 (1981).

12. K.A. Dill and P.J. Flory. Proc. Natl. Acad. Sci. USA 78, 676-680 (1981).

13. M.T. DeEchegaray. Effect on temperature on the elution of non-ionic solutes in micelles containing mobile phases for reverse phase HPLC, Master's Thesis, University of Florida, Gainesville, 1982, p. 8.

14. H.F. Eicke. Surfactants in nonpolar solvents aggregation and micellization. Topics in Current Chemistry 87, 1-83 (1980).

15. B. Lindman and H. Wennerstrom. Micelles amphiphilic aggregation in aqueous solution. Topics in Current Chemistry 87, 85-145 (1980).

16. J.H. Fendler. Interaction and reactions in reversed micellar system. Acc. Chem. Res. 9, 153-161 (1976).

17. K.L. Mittal. "Micellization, Solubilization and Microemulsions," 1, Plenum, New York, 1977, pp. 429-454.

18. J.P. Foley and J.G. Dorsey. Equations for calculation of chromatographic figures of merit for ideal and skewed peaks. Anal. Chem. 55, 730-737 (1983).

19. M. Tang and S.N. Deming. Interfacial tension effects of nonionic surfactants in reversed-phase liquid chromatography. Anal. Chem. 55, 425-428 (1983).

20. D.W. Armstrong and F. Nome. Partitioning behavior of solutes eluted with micellar mobile phase in liquid chromatography. Anal. Chem. 53, 1662-1666 (1981).

21. L.R. Snyder and J.J. Kirland. "Introduction to Modern Liquid Chromatography," 2nd Ed., John Wiley and Sons, New York, 1979, pp. 248-250.

22. Rainin Catalog, 1983, pp. 124-125.

23. L.R. Snyder and J.J. Kirland, "Introduction to Modern Liquid Chromatography," 2nd Ed., John Wiley and Sons, New York, 1979, pp. 374-376.

24. C. Black, G.G. Joris and H.S. Taylor. The solubility of water in hydrocarbons. J. Chem. Phys. 16, 53 (1948).

25. H.F. Eicke and H. Chisten. Is water critical to the formation of micelles in apolar media? Helv. Chim. Acta. 61, 2258-2263 (1978).

BIOGRAPHICAL SKETCH

Maria A. Hernández Torres was born in Mayagüez, Puerto Rico, on May 29th, 1958. She received her elementary and high school education at the Colegio de La Milagrosa, Mayagüez, Puerto Rico. In 1980, she completed her Bachelor of Science degree in chemistry at the University of Puerto Rico Mayagüez Campus. She is now pursuing a Master of Science degree in analytical chemistry at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality, as a thesis for the degree of Master of Science.

John G. Dorsey, Chairman
Assistant Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality, as a thesis for the degree of Master of Science.

James D. Winefordner
Graduate Research Professor
of Chemistry

This thesis was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School, and was accepted for partial fulfillment of the requirements of the degree of Master of Science.

Dean for Graduate Studies and
Research

December 1983